WEST Search History

DATE: Tuesday, May 06, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT; PLUR=YES; OP=ADJ			
L1	flavivirus	550	L1
L2	L1 and ns1	152	L2
L3	L2 and (assay or detection or test)	149	L3
L4	L2 and (assay or detection or test) and dengue	88	L4

END OF SEARCH HISTORY

L3: Entry 112 of 149 File: USPT May 26, 1998

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DOCUMENT-IDENTIFIER: US 5756312 A
TITLE: Immunoreactive polypeptide compositions

Drawing Description Text (4):
FIG. 3 shows a comparison of the amino acid sequences of the putative E2/NS1 region of HCV isolates.

Drawing Description Text (5):
FIG. 4 are graphs showing the antigenicity profiles for the amino-terminal region of the putative HCV E2/NS1 protein (amino acids 384-420), and the gp 120 V3 hypervariable region of HIV-1.

Drawing Description Text (6):
FIG. 5 shows a series of graphs which give the percentage probabilities that a given residue from the amino-terminal region of HCV E2/NS1 protein (amino acids 384 to 420) will be found in either alpha-helix, beta-sheet or beta-turn secondary structural motif.

Drawing Description Text (8):
FIG. 7 shows the deduced amino acid sequences of two regions of the E2/NS1 polypeptide, amino acids 384-414 and 547-647, given for the Q1 and Q3 isolates.

Detailed Description Text (3):
HCV is a new member of the Family Flaviviridae which includes the pestiviruses (Hog Cholera Virus and Bovine Viral Diarrhea Virus) and the Flaviviruses, examples of which are Dengue and Yellow Fever Virus. A scheme of the genetic organization of HCV is shown in FIG. 1. Similar to the flavi- and pestiviruses, HCV appears to encode a basic polypeptide domain ("C") at the N-terminus of the viral polyprotein followed by two glycoprotein domains ("E1", "E2/NS1"), upstream of the nonstructural genes NS2 through NS5. The amino acid coordinates of the putative protein domains are shown in Table 1.

Detailed Description Text (4):
As discussed above, a number of HCV isolates have been identified. Comparative sequence analysis of complete and partial HCV sequences indicates that based upon homology at the nucleotide and amino acid levels, HCV isolates can be broadly sub-divided into at least three basic groups (Table 2). See Houghton et al., (1991) Hepatology 14: 381-388. However, only partial sequence is available for the isolates in group III. Therefore, when the sequences of these isolates are more defined, one or more of these isolates may deserve separation into a different group, including a potential fourth group. Table 3 shows the sequence homologies between individual viral proteins of different HCV isolates as deduced from their nucleotide sequences. It can be seen that the proteins of the same virus group exhibit greater sequence similarity than the same proteins encoded by different virus groups (Table 3). One exception to this is the nucleocapsid protein that is highly conserved among all group I and II viral isolates sequences to date. (In Table 3, the symbol N/A signifies that the sequences were not available for comparison.) For purposes of the present invention, therefore, group I isolates can be defined as those isolates having their viral proteins, particularly E1 and E2/NS1 proteins, about 90% homologous or more at the amino acid level to the isolates classified as group I herein. Group II is defined in an analogous manner. Future groups can likewise be defined in terms of viral protein homology to a prototype isolate. Subgroups can

also be defined by homology in limited proteins, such as the E1, E2/NS1 or NS2 proteins, or by simply higher levels of homology.

Detailed Description Text (5):
It is noteworthy that the putative viral envelope proteins encoded by the E1 and E2/NS1 genes show substantial amino acid sequence variation between groups I and II. Only NS2 exhibits a greater degree of heterogeneity, while the C, NS3, NS4 and NS5 proteins all show greater sequence conservation between groups. The sequence variation observed in the putative virion envelope proteins between groups I and II reflects a characteristic segregation of amino acids between the two groups. An example of this is shown in FIG. 2 where the sequence of the E1 gene product is compared between viruses of groups I and II. The E1 amino acid sequences deduced from nucleotide sequences of HCV groups II and II are shown. In the figure, the horizontal bars indicate sequence identity with HCV-1. The asterisks indicate group-specific segregation of amino acids; the group-specific residues can be clearly identified. Group I sequences are HCV-1, HCT18, HCT23, HCT27, and HC-J1. Group II sequences are HC-J4, HCV-J, HCV J1.1, and BK. Such group-specific segregation of amino acids is also present in other gene products including gp72 encoded by the E2/NS1 gene. FIG. 3 shows the comparative amino acid sequence of the putative E2/NS1 region of HCV isolates which segregate as group I and group II. The latter protein also contains an N-terminal hypervariable region ("HV") of about 30 amino acids that shows large variation between nearly all isolates. See Weiner et al. (1991), supra. This region occurs between amino acids 384 to 414, using the amino acid numbering system of HCV-1.

Detailed Description Text (6):
The putative HCV envelope glycoprotein E2/NS1 may correspond to the gp53(BVDV)/gp55 (Hog Cholera Virus) envelope polypeptide of the pestiviruses and the NS1 of the flaviviruses, both of which confer protective immunity in hosts vaccinated with these polypeptides.

Detailed Description Text (9):
Analysis of biological samples from individuals with HCV induced NANBH indicate that individuals may be carrying two or more HCV variants simultaneously. Two co-existing HV variants were found in the plasma of one individual, J1. In addition, partial sequencing of the gene of an individual with chronic NANBH, who had intermittent flares of hepatitis, revealed that the individual, Q, was infected with two HCV variants (Q1 or Q3). Each variant was associated with only one episode of the disease. An ELISA using a Q1 or Q3 specific peptide (amino acids 396-407) showed that Q developed an antibody response to the Q1 peptide but not the corresponding Q3 peptide, suggesting that Q's recrudescence of disease was due to the appearance of an HV variant. The presence of antibodies to the Q1 peptide but lack of humoral immune response to the Q3 peptide during the second episode of disease suggest that variation in the HV domain may result from the pressure of immune selection. Amino acids 396-407 appear to be subject to the greatest selective pressure in the HV domain. These findings support the thesis that high levels of chronicity associated with the disease might be due to an inadequate immunological host response to HCV infection and/or effective viral mechanisms of immunological evasion. Moreover, they point to the E2/NS1 HV region as a genetic region involved in a viral escape mechanism and/or an inadequate immunological response mechanism(s).

Detailed Description Text (11):
In that the E1 and E2/NS1 regions of the genome encode putative envelope type polypeptides, these regions would be of particular interest with respect to immunogenicity. Thus, these regions are amongst those to which it would be particularly desirable to induce and/or increase an immune response to protect an individual against HCV infection, and to aid in the prevention of chronic recurrence of the disease in infected individuals. In addition, these regions would be amongst those from which it would be desirable to detect HCV variants which are arising during the course of infection, as well as super- or co-infection by two or more variants.

Detailed Description Text (48):
Z is the amino acid sequence of an HCV isolate comprising the above-described VD. Thus, the minimum size of Z is the minimum size of the VD. Z can comprise more HCV

amino acid sequence than just the VD, and can further comprise more than one VD. The maximum size of Z is not critical, but obviously cannot exceed the length of the entire HCV polyprotein. Typically, however, Z will be the sequence of an entire HCV protein (particularly E1, E2/NS1, NS2, NS3, NS4 and NS5) or, even more typically, a fragment of such an HCV protein. Thus, Z will preferably range from a minimum of about 5 amino acids (more preferably about 8 or about 10 amino acids minimum) to a maximum of about 1100 amino acids (more preferably a maximum of about 500, more preferably a maximum of about 400 or even more preferably a maximum of about 200 amino acids maximum). More usually, the polypeptide of formula I and/or Z, when prepared by, e.g., chemical synthesis, is a maximum of about 50 amino acids, more typically a maximum of about 40 amino acids, and even more typically a maximum of about 30 amino acids.

Detailed Description Text (59):
For vaccine applications, it is believed that variable domains from the E1 and/or E2/NS1 domains will be of particular interest. In particular, an E1 variable domain within amino acids 215-255 (see FIG. 2), and an E2/NS1 variable domain within amino acids 384-414 (see FIG. 3), have been identified as being important immunoreactive domains. The preliminary evidence suggests that one or both of these domains may be loci of heterogeneity responsible for escape mutants, leading to chronic HCV infections. Thus, polypeptide compositions as described above where the variable domain(s) in V are one or both of these variable domains are particularly preferred. Furthermore, the polypeptide compositions of the present invention, while particularly concerned with the generally linear epitopes in the variable domains, may also include conformational epitopes. For example, the composition can be comprised of a mixture of recombinant E1 and/or E2/NS1 proteins (exhibiting the variable domains of different isolates) expressed in a recombinant system (e.g., insect or mammalian cells) that maintains conformational epitopes either inside or outside the variable domain. Alternatively, an E1 and/or E2/NS1 subunit antigen from a single isolate that maintains conformational epitopes can be combined with a polypeptide composition according to the present invention (e.g., a mixture of synthetic polypeptides or denatured recombinant polypeptides). In another preferred application for vaccines, the polypeptide compositions described herein are combined with other HCV subunit antigens, such as those described in commonly owned U.S. Ser. No. 07/758,880, entitled "Hepatitis C Virus Asialoglycoproteins" (Attorney Docket No. 0154.002) by Robert O. Ralston, Frank Marcus, Kent B. Thudium, Barbara Gervase, and John Hall, filed on even date herewith, and incorporated herein by reference.

Detailed Description Text (65):
The general techniques used in extracting the HCV genome from a virus, preparing and probing DNA libraries, sequencing clones, constructing expression vectors, transforming cells, performing immunological assays such as radioimmunoassays and ELISA assays, for growing cells in culture, and the like, are known in the art. (See, e.g., the references cited in the "Background" section, above, as well as the references cited at the beginning of this ("Modes of Practicing the Invention") section above.

Detailed Description Text (80):
The immunogenicity of the epitopes of the HCV variable domains, particularly of E1 and E2/NS1, may also be enhanced by preparing them in eukaryotic systems fused with or assembled with particle-forming proteins such as, for example, that associated with hepatitis B surface antigen. See, e.g., U.S. Pat. No. 4,722,840. Constructs wherein the polypeptide containing the HCV epitope from a variable domain is linked directly to the particle-forming protein coding sequences produces hybrids which are immunogenic with respect to the HCV epitope. In addition, all of the vectors prepared include epitopes specific to HBV, having various degrees of immunogenicity, such as, for example, the pre-S peptide. Thus, particles constructed from particle forming protein which include HCV sequences are immunogenic with respect to HCV and HBV.

Detailed Description Text (91):
In another embodiment of the invention, the above-described immunoreactive compositions comprised of a plurality of HCV antigen sets are used to detect anti-HCV antibodies within biological samples, including for example, blood or serum samples. Design of the immunoassays is subject to a great deal of variation, and a

variety of these are known in the art However, the immunoassay will use antigen sets wherein each antigen set consists of a plurality of substantially identical polypeptides comprising the amino acid sequence of an epitope within a first variable domain of an HCV isolate, and the amino acid sequence of one set is heterogeneous with respect to the amino acid sequence of at least one other set. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Detailed Description Text (92):
Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention containing HCV epitopes from variable domains, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, etc) required for the conduct of the assay, as well as a suitable set of assay instructions.

Detailed Description Text (99):
All reactions were performed according to Weiner et al. (1990) Lancet 335: 1-5. M13 sequencing was performed according to Messing et al. (1983), Methods in Enzymology 101: 20-37. The consensus sequence of at least four cloned inserts are presented with the exception of the HCV J1.2 E2/NS1 sequence which was derived from two clones.

Detailed Description Text (100):
Cloning and sequencing of HCT 18 and Th was as reported in Weiner et al. (1991), supra. Nested PCR primers used to clone the amino terminal and carboxy proximal segments of E2/NS1 in patient Q were: PCR I

Detailed Description Text (109):
PCR primers used to clone the HCV J1 E2/NS1 gene were: PCR I

Detailed Description Text (127):
Antigenicity profiles for the HCV E2/NS1 protein and HIV-1 gp120 hypervariable region V3 (aa 303-338) were derived from a computer program based on the degree of sequence variability as originally proposed by Kabat [Sequences of proteins of immunological interest. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health (1983)] for the identification of the hypervariable loops of immunoglobulins multiplied by the average of the individual probability that antibody binding is retained for each possible pair-wise amino acid. Probabilities for retention of antibody binding associated with a given amino acid change were the values experimentally determined by assessing the effects on antibody binding of all possible amino acid substitutions for 103 characterized linear epitopes. Geysen et al., (1988) J. Mol. Rec. 1: 32-41. This algorithm thus weights the variability index to give more significance to amino acid changes likely to have a significant effect on antibody binding, i.e., compensates for conservative amino acid changes. Fifteen HCV sequences [HCV-1, Q3.2, HCT 23, EC10, HC-J1, HCVE1, TH, HCT 27, Q1.2, HCT18, HC-J4, HCV J1.2/HCV J1.1, HCV J , HCV BK], were used to determine the antigenicity profile for HCV. The HIV-1 V3 profile was obtained by averaging 242 individual profiles of 15 sequences selected at random from the numerically greater data base of unique HIV-1 sequences. LaRosa et al., (1990) Science 249: 932-935 & Correction in Science (1991) p. 811. The amino acid sequences of some of these isolates between aa 384 and 420 are shown in FIG. 3.

Detailed Description Text (131):
Comparison of Secondary Structure and Amino Acid Sequence Variation in the HCV E2/NS1 HV and HIV-1 gp120 Domains

Detailed Description Text (135):
Epitope Mapping of the HCV E2/NS1 HV Domain

Detailed Description Text (136):
Overlapping biotinylated 8-mer peptides corresponding to and extending past the E2/NS1 HV domain (amino acids 384 to 416) of HCT 18 (A,D), Th (B,E) and HCV J1 (C,F) were bound to plates coated with streptavidin and reacted with plasma from either HCT 18 (A-C) or Th (D-F). The results are shown in FIG. 6 for HCV isolates HCT 18 (FIG. 6A and 6D), Th (FIGS. 6B and 6E), and HCV J1 (FIGS. 6C and 6F). HCT 18 plasma was diluted 1:200 and Th plasma was diluted 1:500. HVE-1, -2, -3, -4 and -5, represent isolate specific epitopes.

Detailed Description Text (139):
In order to validate antibody binding specificity, antibodies bound to biotinylated peptides containing amino acids 403-407 were eluted and used to block the reactivity of HCT 18 plasma with pins containing overlapping 8-mers for the HCT 18 HV domain. These data indicate that 1) the E2/NS1 HV domain is immunogenic, 2) there are multiple epitopes which map to this region, and 3) a subset of epitopes (HVE-1, -2, -3, -4 or -5 in FIG. 6) in the HV domain are isolate specific.

Detailed Description Text (141):
Determination that Variant E2/NS1 HV Domains Can Be Associated With Flares of Hepatitis

Detailed Description Text (142):
To investigate the possibility of finding HCV variants associated with the intermittent flares of hepatitis often found in chronic HCV infections, we partially sequenced the E2/NS1 gene from a patient, Q, with chronic hepatitis during two distinct episodes of hepatitis approximately two years apart (Q1 and Q3, respectively). The second episode of hepatitis occurred 1.5 years after the termination of interferon treatment.

Detailed Description Text (143):
The differences in the deduced amino acid sequence of the Q1 and Q3 E2/NS1 HV region was strikingly different only between amino acids 391-408 with seven of eight changes occurring between amino acid 398 and 407 (FIG. 7). FIG. 7 shows the deduced amino acid sequences of two regions of the E2/NS1 polypeptide, amino acids 384-414 and 547-647, for the Q1 and Q3 isolates. The amino acid (E) above the Q1 sequence was found in one of four Q1 clones. The boxed amino acids represent the location of the Q1 or Q3 HVE 12mer peptide. Amino acid sequence differences found between Q1 and Q3 are printed in bold type.

Detailed Description Text (144):
Only one amino acid heterogeneity was observed between amino acids 547 and 647 of the Q1 and Q3 E2/NS1 polypeptides (FIG. 7).

Detailed Description Text (145):
To examine the effect of the amino acid substitutions observed in the Q1 and Q3 E2 HV domains on antibody binding, we synthesized a Q1 and Q3 specific 12-mer peptide from amino acids 396 to 407 (HVE Q1 or Q3 in FIG. 7B) and separately reacted the Q1 and Q3 plasma with each peptide in an ELISA. Table 4 shows that antibodies in both the Q1 and Q3 plasma reacted with the Q1 peptide but not with the Q3 peptide. Statistical analysis (Student's Test) indicated that the binding of the Q1/Q3 plasma to the Q1 peptide was significantly above background binding of those plasma to a panel of 12 randomly chosen control peptides ($P<0.001$), while binding of either the Q1 or Q3 plasma to the Q3 peptide was not statistically significant. The data indicate that although patient Q developed antibodies to the HCV Q1 HV domain, which were still detectable two years later at the Q3 time point, no detectable humoral response had developed to the Q3 E2 HV variant which was predominant during the second episode of hepatitis.

Detailed Description Text (147):
Detection of Coexisting E2/NS1 Genes With Distinct E2/NS1 HV Domains in HCV Infected Individuals

Detailed Description Text (148):
FIG. 8A shows the amino acid sequences deduced from two isolates of HCV J1 (J1.1 & J1.2) which were cloned from one plasma sample of the Japanese volunteer blood donor

HCV J1. Kubo et al., (1989) Nucl. Acids Res. 17: 10367-10372. Of the 23 total amino acid changes between HCV J1.1 and HCV J1.2, 9 differences indicated by bold type are clustered in the 30 amino acid E2/NS1 HV domain. Five of the 9 amino acid substitutions in the E2/NS1 HV domain represent nonconservative amino acid changes. Since HCV J1 is the only group II HCV genome which has been cloned in our laboratory, it is unlikely that these differences are due to cross contamination of the HCV J1 plasma. The HCV J1.2 sequence represents a minority sequence in HCV J1's blood since only two E2/NS1 HV variant sequences were identified from 7 cloned sequences which originated from two independent PCR reactions.

Detailed Description Text (149):
Interestingly, a comparison of the HCT27 and HCV E1 isolates (FIG. 8B), which were sequenced in different laboratories and derive from presumably unrelated individuals, showed that the number of amino acid differences in the E2/NS1 HV domain of these isolates were fewer than the number of differences observed between isolates from the same individual.

Detailed Description Text (152):
The immunoreactive compositions of the invention, have utility in the preparation of materials, for example, vaccines, which in turn may be used for the treatment of individuals against HCV infections, particularly chronic HCV infections. In addition, the compositions may be used to prepare materials for the detection of multiple variants of HCV in biological samples. For example, the immunoreactive compositions of the present invention can be used to generate polyclonal antibody compositions that recognize more than one HCV isolate, or as the antigen in an anti-HCV antibody immunoassay. The latter method can be used to screen blood products for possible HCV contamination. Polyclonal antiserum or antibodies can be used to for passive immunization of an individual.

Detailed Description Paragraph Table (1):
TABLE 1 The Putative Protein Domains in HCV
a.a. coordinates (approximate) Protein 1-191
C 192-383 E1 384-750 E2/NS1 751-1006 NS2 1007-1488 NS3 1489-1959 NS4 1960-3011 NS5

Detailed Description Paragraph Table (3):
TABLE 3
Amino Acid Homologies (%) Between Viral Proteins Encoded by Different HCV Isolates

HCV Group	C	E1	E2/NS1	NS2	NS3	NS4	NS5
I compared to I	98-100	94-100	N/A	N/A	N/A	N/A	99-100
II	97-98	77-79	78-81	75-77	91-92	90-93	84-88
III	N/A	N/A	N/A	N/A	86	76-80	71-74
II compared to II	98-100	92-100	89-100	93-100	94-100	97-100	95-100
III	N/A	N/A	N/A	N/A	84	76	74-75
III compared to III	N/A	N/A	N/A	N/A	N/A	91-100	89-100

Other Reference Publication (4):
Weiner et al., "Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins" Virology (1991) 180:842-848.

Other Reference Publication (7):
Kremsdorf et al., "Partial nucleotide sequence analysis of a French hepatits C virus: implications for HCV genetic variability in the E2/NS1 protein" Journal of General Virology (1991) 72:2557-2561.

CLAIMS:

5. A DNA molecule encoding a polypeptide comprising two heterogeneous amino acid sequences from the same variable domain of distinct HCV isolates, wherein the variable domain is selected from the group consisting of the E1 or E2/NS1 domains.